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ROY WALDO MINER

OLFACTION

BY

L. J. MULLINS

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L. J. MULLINS

Few physiological processes remain today as elusive of analysis and as obscure in mechanism as those involved in olfaction. Such a situation is not the result of any dearth of experimental investigations nor because of any reluctance on the part of physiologists to speculate concerning such mechanisms. Rather, we may suspect that our lack of a working hypothesis is to be traced to certain broad gaps in our knowledge of nervous excitation at both the physicochemical and the physiological level. The discussion that follows is not intended to do more than examine the areas of both physics and physiology to which we must look for explanations, and to consider the nature of the difficulties that arise in any attempt at a precise formulation of a theory of olfaction.

Recent reviews that very adequately summarize present-day knowledge of the histology of olfactory cells, the types of phenomena that have been observed, as well as the various theories of olfaction, are available and may be consulted for various details not presented here.[1-5] The olfactory cell is a primary neuron in contrast with the many specialized types of receptors that respond selectively to various physical stimuli. There are about 10 to 20 million such receptors in man[2] distributed over 5 cm.^2 of surface in the upper respiratory passages. This olfactory epithelium, as well as the rest of the nasal surface, also contains bare nerve fibers from the trigeminal nerve and these are generally considered as receptors which signal, by pain, the presence of many types of chemical compounds. The sensitive endings of the olfactory cell are a series of fine hairs (6 to 8 per cell in man, 10 to 14 in the rabbit) with dimensions about 2×0.1 microns. The endings of the olfactory cell are covered with a thin film of fluid, secreted by glands in the epithelium. Presumably this fluid is an ultrafiltrate of blood plasma. The fact that the nerve fibers emerging from olfactory receptors are short and nonmyelinated has discouraged any serious attempt to investigate the phenomena of olfaction by conventional electrophysiological methods. While it seems likely that, in the near future, technical improvements in neurophysiological technique will be such that direct recording in mammals will be possible, certain theoretical considerations, to be presented later, make the interpretation of such direct recording difficult.** One is faced,

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*Aided by a grant (B-139) from the National Institute for Neurological Diseases and Blindness, United States Public Health Service, Bethesda, Md.

**Doctor Lloyd Beidler has advised me that he has been able to obtain direct recording in animals.

therefore, with the problem of interpreting olfactory data without the possibility of testing directly many of the hypotheses that must be advanced. On the other hand, since the nature of the processes whereby chemically inert substances produce nervous excitation are unknown, the possibility exists of improving our understanding of membrane structure, and of mechanisms of drug action by studies of olfactory processes.

Some Physical Properties of Odorous Substances

If we restrict our discussion to chemically inert molecules, it seems quite clear that very small and very large molecules do not have odors. Nitrogen is an example of such a small molecule, and organic molecules with more than 20 carbon atoms are representative of very large molecules. It is sometimes said that the vapor pressure of a substance is important in determining whether it will have an odor or not. Such a rule is certainly misleading. Methane, which is odorless, has a vapor pressure (thermodynamic) of 289 atmospheres at room temperature, while artificial musk, with a distinct odor, has a vapor pressure of about 10^{-6} atmospheres. The explanation often advanced, that odor declines in an homologous series of compounds because the vapor pressures decline, is simply not valid because in practically all the common homologous series, the vapor pressure is considerably above 10^{-6} atmospheres at the point in the series where odor disappears. Correlations between odor and surface activity ignore the fact that many odorous compounds are without such activity.

There is some parallelism between lipoid solubility and olfaction, just as there is some parallelism between narcosis and lipoid solu-

TABLE 1

THRESHOLDS FOR OLFACTION

Substance	*Concentration mg./liter*	p_{olf} *atm.* $\times 10^{-7}$	p° *atm.* $(37^{\circ}$ *C.)*	A_{olf} $\times 10^{-3}$
Amyl acetate	0.039	90	0.013	1.
Ethyl acetate	0.69	1,760	0.23	1.
Butyric acid	0.009	18	0.003	1.
Ethyl ether	5.83	13,000	1.1	1.
Chloroform	3.30	6,700	0.4	2.
Art. musk	0.00004	0.029	0.0000013	2.
Carbon tetrachloride	4.53	6,700	0.24	3.
Valeric acid	0.029	50	0.001	5.
Amyl alcohol	0.225	500	0.007	7.
Nitrobenzene	0.146	300	0.0007	40.
Methyl salicylate	0.10	150	0.00026	60.

bility. A generalization of this latter notion has led Ferguson[6] and Brink[7] to use the thermodynamic activity of a substance as a measure of its pharmacological effectiveness in causing narcosis. Such treatment has enabled one to predict, with considerably accuracy, the thermodynamic activity of any given substance that is required for narcosis (A_{nar}). The validity of this treatment rests with the assumption, apparently fulfilled for narcosis, that the process is an equilibrium phenomenon. Some improvement in the Brink rule of "equal degrees of narcosis at equal thermodynamic activity" has been made[8] by introducing the requirement that narcosis take place at constant volume fraction of the narcotic dissolved in the membrane. One can then account for the shape of the curve relating threshold activity for narcosis (A_{nar}) to chain length in a homologous series by the use of an expression* that relates the cohesive energy density of a liquid to the logarithm of the activity coefficient that a given substance will show in membranes of various cohesive energy densities. From an analysis of a variety of data, it can be shown that the cohesive energy density of the membrane is different for various regions of the nervous system. The order of these "solubility parameters" is such that brain and synapses have a low, while peripheral nerve has a much higher value. The success of such treatment leads one to inquire whether data for thresholds for olfaction expressed as thermodynamic activities (A_{olf}) might be treated profitably by similar methods. To change the data for olfactory thresholds to activities we have only to obtain figures for the partial pressure of a given substance that yields threshold olfaction, and divide by the vapor pressure of the pure liquid at 37° C; thus $p_{olf}/p^{\circ} = A_{olf}$, where p° is the vapor pressure of the pure liquid. Such a series of thresholds has been calculated from the data of Allison and Katz,[10] and TABLE 1 shows that indeed the threshold expressed as A_{olf} is much more constant than that expressed as weight per unit volume. While these data indicate that a threshold A_{olf} of about 10^{-3} appears to be common, the molecules chosen for test are not very similar, and an homologous series of compounds is more suitable for purposes of analysis. If data for such series are examined, the apparent constancy of A_{olf} as shown above diminishes. Some allowance must be made for the fact that, while the data for narcosis can be analyzed on the basis that narcosis is an equilibrium

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*For dilute solutions: $ln\ \gamma = V_m\ (\delta_{nar} - \delta_{mem})^2\ /RT$, where $\gamma = A_{nar}/X_{nar}$, and X_{nar} is the mole fraction of narcotic in the membrane. V_m is the molal volume of the narcotic molecule (cc./mole), δ_{nar} is the *solubility parameter* of the narcotic and equals $(\Delta E^v/V_m)^{1/2}$ where ΔE^v is the energy of vaporization of the liquid. δ_{mem} is the solubility parameter of the membrane, or (cohesive energy density)$^{1/2}$ of the membrane molecules. We use solubility parameter or cohesive energy density interchangeably.

This method of analysis is roughly equivalent to saying that a variation in δ_{mem} means that the membrane has a variable partition coefficient. Low values correspond to a paraffin oil membrane and high values to an alcohol-like membrane.

process, there is no suggestion either from experimental data or from theoretical considerations that olfaction is at all similar to an equilibrium process. We might hope that olfaction is some kind of steady-state phenomenon but, as we shall see later, there are reasons to doubt the persistence of even this somewhat more complex situation except for over very short periods of time. There is a process, however, that follows olfactory stimulation that may be considered to be an equilibrium effect. This is "adaptation" or the cessation of odor sensation after some seconds even at threshold.

The Purity of Reagents

Since it is possible to detect by smell certain organic, chemically inert substances at a value of A_{olf} of about 10^{-6}, it is obvious that the purity of reagents used for experimentation is of paramount importance. The requirements for purity are especially severe when one is dealing with substances that are, themselves, rather weak olfactants. For many organic compounds, a purity of 99 per cent is high, but it can be appreciated that if the 1 per cent impurity had a threshold of 10^{-6} while the substance itself had a threshold of 10^{-4}, the two odors would be of equal intensity. The preparation of reagents with an impurity of 1 part in a thousand is not particularly difficult, but 1 part in 10,000 represents, for certain classes of compounds, the highest purity obtainable. For reasons connected with purity, the odors assigned to a variety of organic substances must be regarded with some skepticism. Examples of errors of this sort abound. Moncrieff[3] has summarized experience with the normal paraffin series by saying that while pentane has an indistinct odor, higher members of the series, especially octane, have a powerful petrol smell. Our observations (to be presented later) are completely at variance with this statement in that octane has a very weak odor, not noticeably different from pentane. The explanation of this difference must lie in the fact that a petrol or gasoline smell is associated with various types of ring hydrocarbons which are removable only with difficulty from the n-paraffins. If paraffins are prepared not from petroleum but from a pure parent compound, they do not smell at all like gasoline. Similarly, the odor of acetylene is mainly that of impurities such as phosphine, and the anomalous "pungent" odor of allyl alcohol is due to allyl formate[11] present as an impurity of synthesis. For reasons such as these, confidence in associating a particular odor with a given chemical compound can come only from a determination that either the threshold for olfaction for a particular substance is very low, or that the odor results when the particular substance is synthesized by a variety of organic reactions that use different starting materials and hence form different impurities. While the information is probably available in the form of experience, there does not appear to be any literature on this point.

The Measurement of Olfactory Thresholds

Assuming that the requirements of reagent purity have been met, there remains a very considerable technical problem in the measurement of "thresholds" for olfaction. Since such values ought to represent a measure of the effectiveness of a given compound in causing olfaction, some effort should be made to give a precise definition to the term. Assuming that an odorless inert gas is being forced to flow through the nose at a rate of about 5 liters per minute, the introduction of an odorous substance at such a rate that its concentration rises from zero to five thresholds in about one minute will not give any sensation If the odorous substance is introduced in such a manner that its concentration rises as rapidly as possible in the air stream, a maximum sensitivity with respect to its detection is obtained. There is some variation in opinion regarding the question of whether threshold is reached when a subject can tell whether there is "something there" or rather when he can identify the substance being used for test. We have made a few preliminary experiments on this point. If the oral cavity of a subject is ventilated with various substances while the nose is held closed, a series of thresholds for the detection of substances in the mouth can be obtained. The paraffins are poorly detected, as compared with olfactory sensitivity for these substances, the alcohols appear intermediate, and the chloroparaffins are detected about equally well by mouth and by nose. Since our experimental arrangement is unable to stimulate selectively the olfactory epithelium, measurements of threshold will be complicated by the factor of trigeminal and taste stimulation. Only in those cases where it can be demonstrated that the olfactory threshold is greatly lower than any other will threshold results be entirely unambiguous. From the foregoing, it would appear that the olfactory threshold ought to be defined in terms of the lowest concentration of a substance that still permits of its recognition and differentiation from other odorous substances.

In the measurement of olfactory thresholds, some controversy has settled about whether to use "sniffing," which ventilates the olfactory epithelium to a variable extent, or continuous ventilation, which is supposed not to be physiological. Such disagreement, it would seem, can be resolved by studying the variation in olfactory threshold at different velocities of air flow through the nose. When this is done, it appears that for monorhinal flows of greater than about 4 liters per minute, the threshold is rather constant. We have made such measurements for n-butane and they are shown in FIGURE 1. Flows higher than about 6 liters per minute give rise to unpleasant and distracting effects in th nasal mucosa and lead to greater variability in the measured thresholds. The problem of loss of odorous substance by absorption in the mucosal surfaces before the air stream reaches the olfactory epithelium must also be considered. Such an effect undoubtedly exists, but high velocities of gas flow will minimize such loss. We hwe used monorhinal stimulation because of the greater certainty of a known gas flow, and because subjects consider this arrangement more comfortable.

Cur olfactometer consists of a series of capillaries across which the pressure drop has been calibrated to give gas flow. Suitable capillaries are selected to give the range of vapor dilutions required, and water-pumped nitrogen is used as a diluent gas. Since it is technically rather difficult to obtain reliable flow dilutions of more than about 1:1000, we have cooled the odorous liquid in some instances to −78° C. in order to achieve a preliminary reduction in vapor pressure of about 300 times.

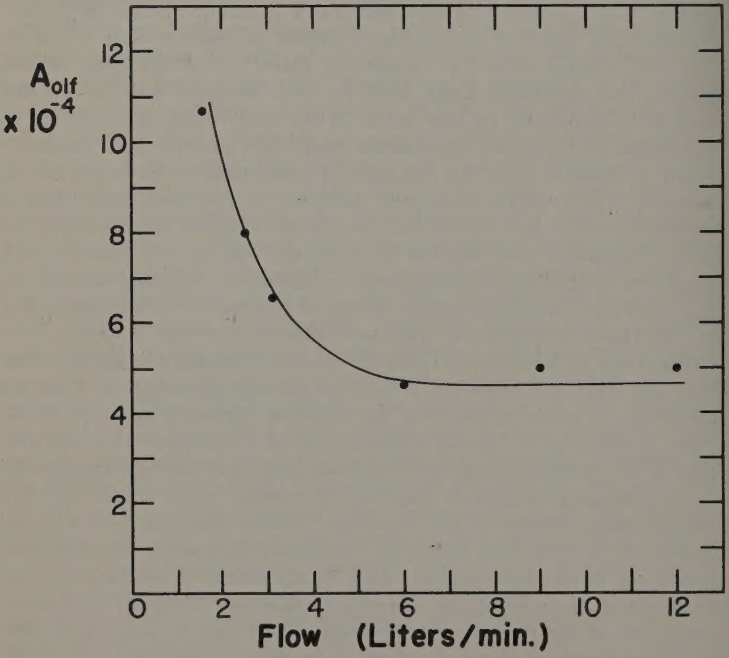


FIGURE 1. The dependence of the threshold for olfactory stimulation by n-butane upon the rate of flow of gas mixture (monorhinal) is shown for flows of from 2 to 12 liters per minute.

This method is a satisfactory one for obtaining high dilutions, but great care must be exercised to insure that liquid spray is not carried over into the warm part of the flow apparatus, an effect that may account for some of the low threshold values reported in the literature.

Perhaps the best test of threshold is the reproducibility of the measurements. We have not investigated the time relationships involved in the recovery of olfactory sensitivity, but we have routinely given subjects a 1-second stimulus with odor, and allowed a 10-second rest period. Under these conditions, subjects can maintain olfactory detection unimpaired almost indefinitely. Threshold conditions are manifest when a subject begins to find difficulty in recognizing every stimulus.

The Duration of Odors

We have made an attempt to verify the observations of Woodrow and Karpman,[20] who claimed that the duration of an odor sensation was directly proportional to the concentration of a substance in air. Our study involved the use of 23 subjects who were exposed to odor concentrations in the range of 1 to 100 times threshold. Some of the results, shown in TABLE 2, indicate that it was not possible to find any correlation between odor duration and odor concentration. Rather, there appears to be a correlation between the duration of an odor and the molecule that initiates the odor. The subjects found this type of study difficult because of the problem involved in deciding when olfactory stimulation had ended and merely trigeminal stimulation was left. To get around this confusion, a musk (pentadecanolide) was used as a substance

TABLE 2

ODOR DURATIONS

Substance	*Concentration (thresholds)*	*Mean odor duration (minutes) for Subject No.*							
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
n-Butane	1-4	.07			.05		.07		.10
	5-9	—	.20	.07	.10	.30	.07	.20	.15
	10-20	.12	.24	.07	.10	.30	.05	.33	.15
	21-100	.13		.06					.15
n-Butyl alcohol	1-5	.30		.11	.08			.12	.24
	5-9	.34	.32	.13	.06	.42		.10	.25
	10-20	.27	.33	.12	.06	.42			.20
	21-100	.20	.46		.06				
Pentadecanolide	1-4	.18		.07	.10		.18		.20
	5-9	.18		.09	.10	.15	.18		.19
	10-20	16	.12	.09		.18	.20		

for odor duration studies since it is a characteristic of large molecules of the sort used in perfumery that they do not initiate trigeminal stimulation. This substance gave much more uniform results with all subjects, although the results are preliminary in that a technical problem of the tendency of musk to adsorb on glass may have introduced some error into the concentration figures. Again, however, it was not possible to demonstrate any dependence of odor duration on concentration. A partial explanation of the results of Woodrow and Karpman may be found in the fact that they used flow velocities of 1 liter per minute. This low flow makes it probable that, since a portion of the odorous material must be absorbed by the mucous membranes of the nose before the stream reaches the olfactory epithelium, what was being observed was a continuous increase in concentration with time at the olfactory epithelium.

It would be of great assistance in evaluating measurements from different laboratories if a standard test substance were used for threshold measurements. Our experience would suggest that n-butane is suitable for such a purpose for a number of reasons. In the first place, it is a gas that can be obtained in very high purity; it is most unlikely to be contaminated in the laboratory, as it can be led directly from the cylinder into the olfactometer; and it has a reasonably low threshold, one that subjects report as giving a clear indication of threshold without the distraction of trigeminal or taste stimulation.

Olfaction at Constant Membrane Concentration

It would seem that one of the most profitable methods of attack upon the problem of mechanisms in olfaction would be to have at hand a series of measurements for the thresholds for olfaction within a variety of homologous series of liquids of different cohesive energy densities, because such measurements would permit some inference as to the nature of the internal attractive forces between the molecules of the receptor membranes themselves. We have measured thresholds for olfaction (A_{olf}) in man, therefore, for the n-paraffins, the n-alcohols, and the 1-chloroparaffins. The paraffins have a low cohesive energy density ($\delta = 7$), the chloroparaffins an intermediate ($\delta = 8.5$), and the alcohols a high and variable solubility parameter ($\delta = 14.5 - 9.0$). The paraffins used were the highest purity obtainable and were prepared as standard samples for the American Petroleum Institute,* from known pure starting materials. The alcohols and chloroparaffins were subjected to purification and precision fractional distillation in the laboratory. The results obtained are shown in FIGURE 2, in which the activity for threshold olfaction is plotted against the number of carbon atoms in the chain.

*Pittsburgh, Pa.

The dotted line on the curve shows the approximate thermodynamic activity required for the narcosis (A_{nar}) of man and laboratory animals by the paraffins and chloroparaffins. It is thus clear that, while small and large molecules may have thresholds for narcosis and olfactory stimulation that are similar, molecules of a size represented by 4 to 5 aliphatic carbon atoms have optimal characteristics for olfactory stimulation. A curve very similar to that shown in FIGURE 2 has been obtained by Dethier for chemoreception in insects both for the alcohols[21] and the aldehydes.[22]

The shape of the curve in FIGURE 2, from the 4 carbon-atom-molecule on, cannot be accounted for by the assumption that all paraffins must reach a given concentration in a receptor of cohesive energy density similar to that of the paraffins ($\delta = 7$). Nor can the points for the alcohols be explained by assuming that such substances seek a receptor similar to alcohol cohesive energy densities. A receptor membrane somewhere between the alcohols and the paraffins would give a rough fit, but then the 1-chloroparaffins ($\delta = 8.5$) would not. The data are best fitted if a receptor $\delta = 12$ is selected as the type that determines whether stimulation by the paraffins and chloroparaffins will occur, and a receptor $\delta = 8$ for the alcohols.

Although the limiting receptor-type for the paraffins is apparently $\delta = 12$, much greater concentrations of paraffin will be developed in $\delta = 8$ receptors. The suggestion then appears that (1) adaptation to odor is equivalent to the narcosis of some of the $\delta = 8$ receptors; and (2) olfactory stimulation requires the activity of the $\delta = 8$ and $\delta = 12$ receptors in concert.

In postulating a parallelism between narcosis and adaptation to odor, the question arises as to whether volatile anesthetics always have an odor. In the case of Xenon, which is capable of producing general anesthesia in man at partial pressures of 500 mm., the answer is no, and a similar case is that of nitrogen narcosis at pressures of greater than one atmosphere. Methane is often cited as an example of an odorless gas, but since the vapor pressure (fugacity) of methane at 37° C. (thermodynamic) is about 500 atmospheres, its activity at 1 atmosphere of pressure is only 2×10^{-3}, hence we can say only that A_{olf} is greater than this value, as it seems unlikely that either subjects or experimenters will be found who will care to test compressed methane-oxygen mixtures at 10 or more atmospheres of pressure. The fact that small gas molecules have either no odor or very weak odors cannot be due to activity coefficient deviations in the membrane, because these small molecules are all effective as narcotics at normal values for A_{nar}. The implication from the shape of the curve in FIGURE 2 for C-1 to C-4 is that it is possible to dissociate olfaction from narcosis if one uses very small

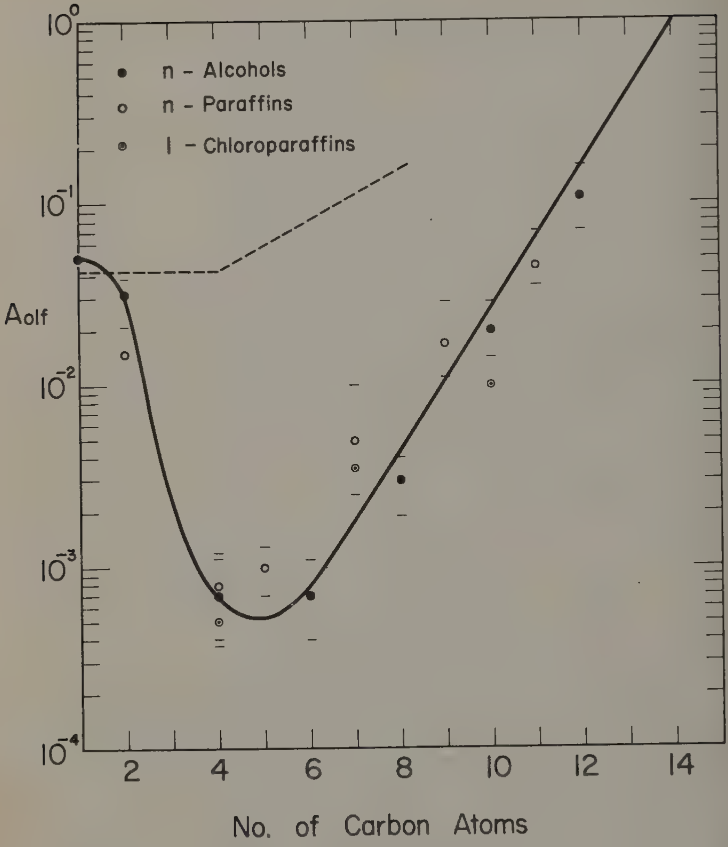


FIGURE 2. The olfactory thresholds for three homologous series of compounds (alcohols, paraffins, and chloroparaffins) are plotted against the number of carbon atoms in the compound. The horizontal lines above and below the alcohol and paraffin points represent standard deviations. Six subjects were used, and the data represent three or more presentations to each, at more than 24-hour intervals. The purity of the paraffin samples was as follows: ethane, 99.78; butane, 99.79; pentane, 99.95 ± .04; heptane, 99.99 ± .01; nonane, 99.92 ± .06; undecane, 99.96 ± .03 mole per cent. The alcohols and chloroparaffins were purified so that samples agreed with published refractive index values in the fourth decimal place. The dotted line shows the threshold for narcosis of man and laboratory animals by paraffins and chloroparaffins.

molecules, and that a threshold value of molal volume of about 100 cc. per mole (butyl alcohol) appears to exist for optimal olfactory effect.

Cross-Adaptation Experiments

If the olfactory epithelium can be divided into groups of receptors with different membrane cohesive energy densities, it ought to be possible to map the distribution of these receptors by examining the extent to which substances of various cohesive energy densities interfere with the threshold of other substances of different δ. Experiments have been performed where a subject was adapted to a paraffin at a concentration 10 times threshold, and when he reported that the odor had disappeared, he was tested for threshold with an alcohol. The results of such experiments on three subjects are shown in TABLE 3.

It would appear that since butane does not disturb the threshold for butyl alcohol and vice versa, there are at least two types of receptor membranes with different solubility parameters. In addition, the effect of acetone in influencing the butyl alcohol threshold more than the butane threshold would suggest a third type of receptor, as does much other evidence to be presented later. We have therefore included in TABLE 2 a computation of the mole fractions of the various adapting substances that may be expected in membranes of $\delta = 8$, 10, and 12. A study of data for narcosis reveals that 12 is a common value for peripheral nerve membranes, and that 10 is close to values for synaptic membranes, while values as low as 8 have not been noted. Since butane is represented mainly in the $\delta = 8$ receptors (although stimulation is limited by the number of $\delta = 12$ receptors that can be activated) and butyl alcohol in the $\delta = 10$ and 12 receptors, the effect of actone in interfering with the thresholds of these substances is understandable.

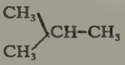
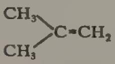
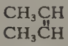
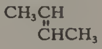
TABLE 3

CROSS-ADAPTATION MEASUREMENTS

Olfactory Cells adapted at 10 × threshold to	δ	*Threshold measured for*	δ	*Change of threshold %*	*Calculated equilibrium mole fraction (× 10^{-4}) of Adapting Molecules in Receptors of*		
					$\delta = 8$	$\delta = 10$	$\delta = 12$
Butane	7	Butyl alcohol	11	0	100	10	1
Butyl alcohol	11	Butane	7	0	10	100	100
Acetone	10	Butane	7	100	20	100	20
Acetone	10	Butyl alcohol	11	500	20	100	20

TABLE 4

OLFACTORY THRESHOLDS FOR FOUR CARBON-ATOM-MOLECULES

Substance	*Structure*	A_{olf} × 10^{-6}	*Std. Deviation*	δ	V_m cc./mol.	*Purity mole %**
n-Butane	$CH_3(CH_2)_2CH_3$	700	100	6.7	101	99.78
2-Methyl propane (isobutane)		120	10	6.3	105	99.88
2-Methyl propane (isobutylene)		500	75	6.7	94	99.44
Butadiene-1,3	$CH_2=CH-CH=CH_2$	20	3	7.1	88	99.84
Butene-1	$CH_3CH_2CH=CH_2$	4	1	6.7	95	99.40
cis-Butene-2	 CH_3CH $\|\|$ CH_3CH	4	1	7.2	91	96.18
trans-Butene-2	 CH_3CH $\|\|$ $CHCH_3$	350	35	7.0	91	99.92

*By mass spectrometer analysis. Principal impurities are given in parentheses after each substance. Butane (isobutane), isobutane (butane), isobutylene (butane), butadiene (butene-2), butene-1 (isobutane, butane), *cis*-butene-2 (*trans*-butene-2), *trans*-butene-2 (*cis*-butene-2).

The Effect of Molecular Shape on Olfactory Thresholds

There is abundant evidence that molecular shape affects the qualitative nature of odors,[16] but there is little information as to the possible effects on threshold that shape factors may have. In solutions, the effects of unsaturation, or of special steric configurations are usually small in so far as they influence thermodynamic deviations from ideality, but in an oriented liquid such as the receptor membrane appears to be, geometrical factors may be expected to be of much greater importance. Our discussion, up to this point, has concerned completely flexible molecules (paraffins, 1-chloroparaffins, and n-alcohols) all of which have rather indeterminate shapes. By this is meant that since the molecules are so constructed that free rotation is permitted about each carbon-carbon bond, they can assume shapes varying from a cylinder to approximately a sphere. In the gas phase, we may expect a large number of different shapes to exist and, in the liquid state, or in solutions, the molecules will assume those shapes that correspond to conditions of minimum energy. The physical situation in solutions is too complex to analyze in so far as shape is concerned, and is therefore generally neglected in theoretical treatments that employ spheres as models of molecules in liquids and solutions. Molecules can be made "rigid" in a number of ways: (1) by introducing double or triple bonds, which prevent free rotation; (2) by branching such that, although free rotation is possible, no change in shape is produced (as is *tert*-butyl); (3) by the introduction of substituents into paraffins such that there are steric or electrostatic hindrances to free rotation (as in 1, 2 dichloroethane); and (4) by ring formation. If we are to test the effects of molecular shape on olfactory thresholds, the following requirements for test substances ought to be met: (1) all substances should have the same δ and V_m; (2) substances should present a wide variety of invariant shapes. Such a group of substances appeared to be n-butane and its various unsaturated derivatives. All these substances have boiling points at about 0° C., and have very similar molal volumes and cohesive energies. The thresholds measured for these substances are shown in TABLE 4. Several features are worth mentioning. If the olfactory effect of unsaturated molecules were related to some sort of chemical reactivity of the double bond, butadiene might be expected to be a hundred or so times as stimulating as any of the butenes, but such is not the case. Isobutylene might be expected to be the most active olefin, but its threshold is not particularly different from that of n-butane. The data were obtained from three to four presentations to each of nine subjects. The most remarkable difference in threshold is that observed between the two geometrical isomers of butene-2, with the *cis*- form being about 100 times as effective in stimulating olfactory cells as is the *trans*- form.

Any really quantitative treatment of these effects must await some sort of mathematical method of representing molecular shapes, and some sort of physical characterization of the cell membrane. Lacking these, we can only look at models of the molecules and try to decide about the types of shapes that seem to favor olfactory stimulation. In FIGURE 3 are shown models of the various molecules used, together with figures indicating the olfactory stimulating power ($1/A_{olf}$) of the molecules relative to butane. In general, there are three groups of molecules. In the first, with a weak stimulating power, are n-butane, isobutane, isobutylene, and *trans*-butene-2. The last three molecules have completely specified shapes, and the difference in threshold between butane and isobutane might be assumed to be the result of reducing the number of configurations of the olfactant molecule and hence making more molecules effective for penetration. Butane is shown in the photograph in a coiled configuration, but it can exist, of course, in a number of possible shapes, and it is conceivable that many of these shapes are not effective in causing membrane excitation, or are not effective in penetrating into the membrane. It might be remarked that narcosis is not particularly sensitive to molecular configuration, at least for molecules with small volumes* (for mice, propane is a general anesthetic at $A_{nar} = 0.07$, cyclopropane at 0.03), and this fact suggests that it may not be so much hindered penetration as improper shape for optimal excitation that is involved. The cylindrical molecule, *trans*-butene-2 appears to have about as much olfactory effect as butane; that is, its shape is not a configuration that is specially adapted to stimulation. Butadiene is a substance that has a threshold intermediate between the high and low threshold substances. Since this is a conjugated diene, free rotation about the middle bond in the molecule is hindered and the compound can exhibit the equivalent of geometrical isomerism. It has been estimated[17] that in the gas phase at 20° C. there is an equilibrium with 96 per cent of the molecules in the *trans*- form. While we might imagine that in the liquid the equilibrium is only 80 per cent *trans*-, so that the *cis*- form fraction would be sufficient to yield our measured threshold, this is not necessarily so. The notion that the threshold of mixtures of odorous substances with similar cohesive energies can be predicted from per cent composition of the strongest olfactory stimulant, even if the mixture is an ideal solution, requires experimental investigation. We have shown butadiene, in FIGURE 3, in the *cis*- form on the assumption that it is this form that stimulates olfaction. A third group of compounds includes butene-1 and *cis*-butene-2. The former is not completely rigid, while the latter is, and the shapes do not appear to be greatly similar. If these four carbon-atom compounds are classified on the basis of similarity of odor, we have the following: (1) butane, isobutane; (2) *trans*-butane-2; (3) isobutylene (very weak), butene-1, *cis*-butene-2, and butadiene. On the basis of shape, the prediction was made that butyne-2 should have an odor and threshold similar to that of *trans*-butene-2, and this prediction was verified by experiment. The odors are reported as similar, and the thresholds are 2.3×10^{-4} (butyne-2) and 3.5×10^{-4} (*trans*-butene-2). A further test of the analysis already given was to measure the value of A_{olf} for the perfectly planar molecule ethylene. The value obtained was 2×10^{-5}, and the odor was judged similar to that noted previously for planar molecules. This threshold contrasts sharply with A_{olf} for ethane (2×10^{-2}). Both gases were mass-spectrometer analyzed reagents of 99.9+ per cent purity. No actual impurities were detected, but 0.04 per cent CO_2 may have been present in the ethylene. Our choice of reagents for testing shape-threshold

*The development of gaseous general anesthetics is of interest in this connection. Nitrous oxide and ethylene at atmospheric pressure both produce a weak anesthesia with little excitement and are rigid molecules. Cyclopropane produces stage 4 anesthesia but not without some excitatory disturbances, principally on cardiac rhythm. Attempts to obtain still more powerful anesthetic agents in the rigid 4-carbon-atom or larger paraffins have been uniformly unsuccessful. These substances are more powerful, but they have associated with their anesthetic action excitatory effects on either the central nervous system or the heart. Ideal anesthetics would thus appear to be small molecules, hence those with a minimum of membrane excitatory (or olfactory) effect.

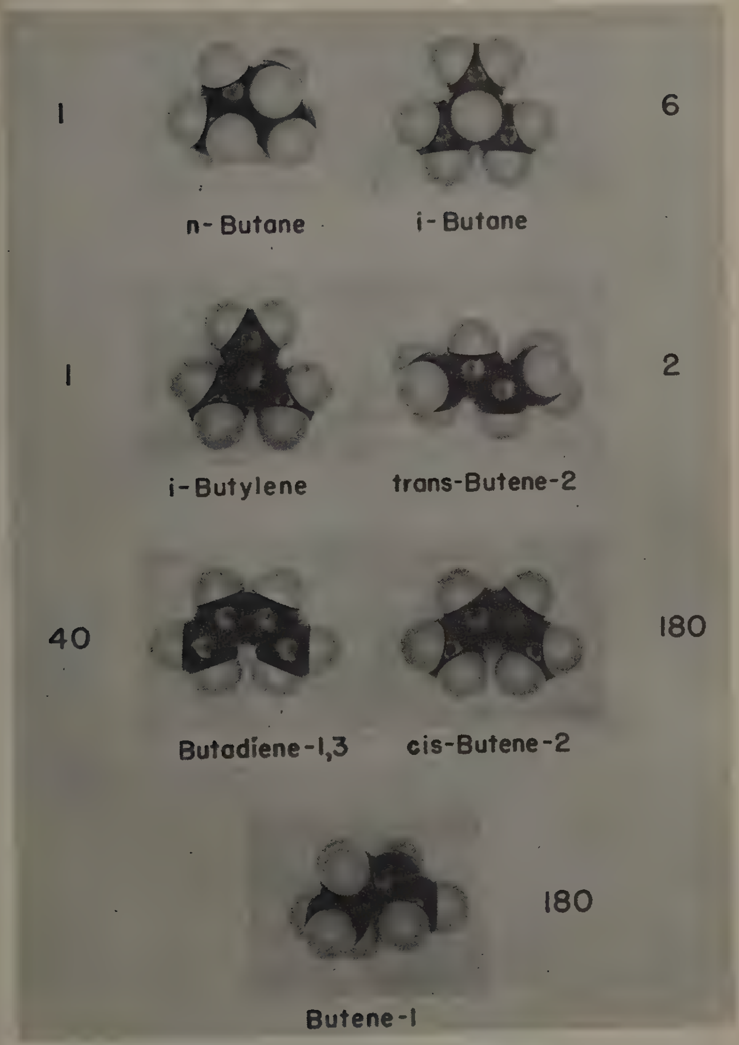


FIGURE 3. Stuart models of the various four-carbon-atom molecules used in olfactory measurements are shown, together with a figure representing the olfactory stimulating power $(1/A_{olf})$ relative to n-butane = 1. The photographs have been taken to show mainly a side view of the molecules.

relationships is somewhat limited by the availability of materials of proved purity. Work is under way at present to extend the scope of these measurements.

Discussion

Membrane excitation by chemical substances. There is evidence[12-14] that narcotics, acting at concentrations lower than threshold for narcosis, exert a stimulating influence on nervous structures and, indeed, this action is one of the more undesirable ones in the use of many agents as anesthetics. There are chemical agents that will cause a decrease in the threshold of excitable tissues to the point where self-excitation will occur. If such substances are injected into animals, they will sometimes produce an excitation of the motor cortex and hence have convulsant action. Camphor is such a substance, and it is also, interestingly enough,

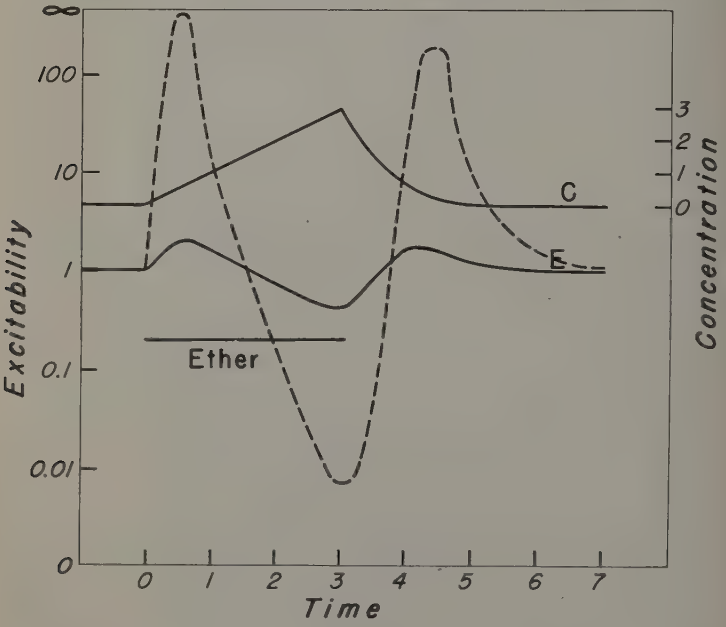


FIGURE 4. A plot of both excitability versus time and membrane concentration of ether versus time. The concentration curve rises linearly with time when ether is applied as shown by the bar, and the excitability for single nerve fibers (Arvanitaki) at first rises and then falls. The dotted line is the postulated change in the excitability of an olfactory cell as ether is applied. Time is in arbitrary units.

an olfactory stimulant. An example of the excitatory action of common anesthetics is provided by the study of Arvanitaki,[13] in which chloroform and ether caused a marked decrease in the threshold for electrical excitation of single nerve fibers before a final increase in threshold. The effect was perfectly reversible, and the durations of the decreased and increased thresholds were about equal. If a chemical agent lowers the threshold to the point of self excitation before finally causing narcosis, we have what would appear to be an acceptable model for certain aspects of olfaction. In FIGURE 4 we have plotted both the data of Arvanitaki and a modification designed to permit the self-excitation of the olfactory receptors when a certain mole fraction of chemical agent has been reached in their membranes.

Since we have postulated a number of different types of receptors, it is apparent that the possibilities for their excitation by chemical substances will be numerous. It is practically impossible to deal with all the ways that this ensemble of receptors could be excited, and we have limited the discussion to a particular case. Our experiments with adaptation are not extensive enough to construct a precise plot of number of receptors *versus* their solubility parameter, and the diagram shown in FIGURE 5 has been made on the assumption that there are somewhat fewer $\delta = 10$ receptors than those of the other two classes. There may be other types, but a great number of the phenomena of olfaction can be dealt with by using this somewhat simplified model. The dotted lines on the chart indicate the approximate limits, with respect to the solubility parameter, of substances that can be shown to initiate olfaction. On the basis that both butanol and butane have approximately the same value for A_{olf}, we have set the distribution of 8 and 12 receptors as equal. The number of 10 receptors has been set somewhat lower because butyl chloride has about the same threshold as these other substances and has a value of 8.5 for solubility parameter, which favors its entrance into a large number of receptor types.

Qualitative differences in odor. There are difficulties with the assumption that odorous substances are merely subjected to a sorting out process by the olfactory epithelium such that molecules are distributed to the membranes of those receptor cells with the greatest similarity in cohesive energy density. In the first place, it is hard to see how very many different odors could be recognized on this basis, and it is also difficult to imagine how a chemical substance at widely varying concentrations is usually recognizable as a specific odor. A considerable improvement in this proposed signaling system can be made if the requirement is introduced that a substance that is to produce an odor must stimulate all three receptor types simultaneously. In this case, the central nervous system is required to analyze only differences in time

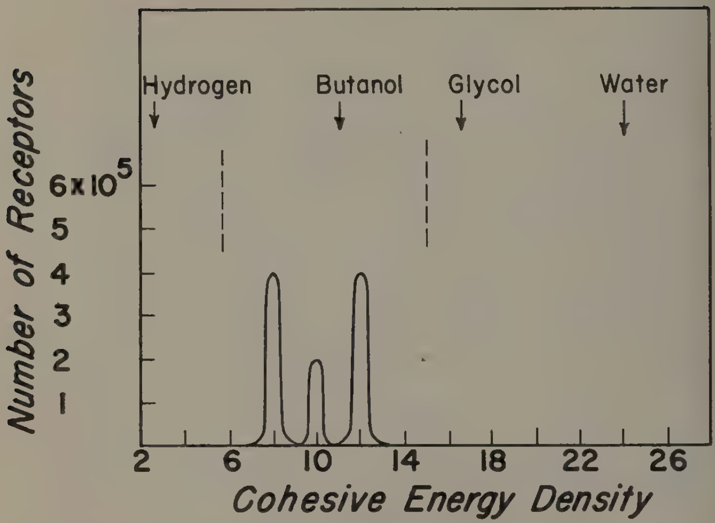


FIGURE 5. The number of olfactory receptors is plotted against the cohesive energy density$^{1/2}$, or the solubility parameter of the receptor membranes. Values for δ are given at the bottom of the chart for a variety of substances, and the dotted line $\delta = 5.5$ to 15 indicate the approximate limits for the olfactory detection of chemically inert substances.

between the start of excitation in the various types of receptors in order to detect specific odors. The physical problem then becomes not one of equilibrium considerations, but a problem of the prediction of the kinetics of uptake of molecules by receptors of varying δ. An illustration of how this proposed system would work is given in FIGURE 8, in which a substance of $\delta = 8$ is being presented to the olfactory epithelium at perhaps 10 times threshold. The assumption with respect to kinetics is that molecules enter and excite most easily those receptors with similar cohesive energies. The slopes of the uptake curves are all represented as linear with time because the receptors are all far from equilibrium. The excitatory levels of the membranes of various receptors are represented as normal, self-excitation, normal, and narcosis in accordance with FIGURE 6. The stimuli resulting from single receptors (shown at the top of the chart) have been drawn such that the length of the train of impulses is governed by the time taken for the olfactant concentration in the membrane to go from one to two units. One point that might be mentioned relative to whether adaptation to odor is central or peripheral is the following: in experimental investigations, where recording is done from the olfactory bulb, there are indications that there is electrical activity in the presence of subthreshold odor concentrations.[15] This is to be expected from the above model because we are unable to specify the threshold for the stimulation of a single type of receptor. Olfaction must always require perhaps 10 to 100 times the activity that is required for the stimulation of single receptor types.

The n-alcohols may be selected as examples of how one may interpret qualitative differences in odor. There appears to be general agreement that alcohols C_1 - C_3 have similar odors, as do C_4 - C_5 while a third group is represented by C_6 - C_8.[3] We have tabulated the δ values for the n-alcohols (TABLE 5), together with an analysis of the sequence of receptor stimulation to be expected from each alcohol as judged by the similarity of alcohol δ to receptor δ. While there will be differences in time of stimulation, and differences in membrane concentration due to the increase in molecular volume as one ascends the series, it appears

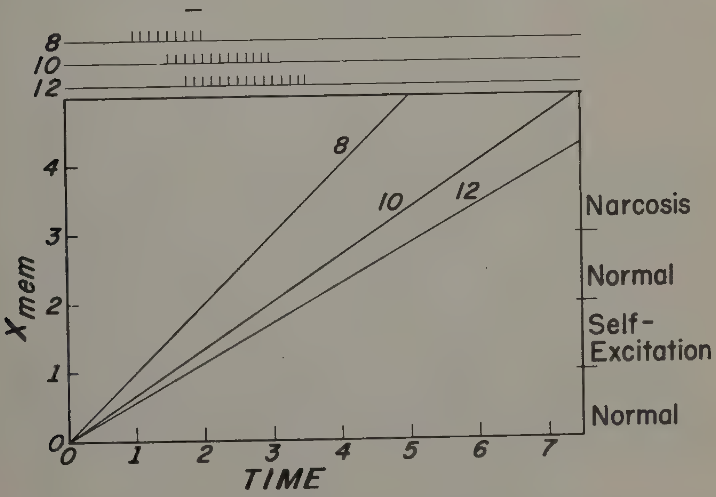


FIGURE 6. A plot of the increase in membrane concentration with time in single receptors 8, 10, and 12 for a substance with a solubility parameter of 8 presented at about 10 × threshold. The lines at the top of the chart indicate the electrical activity to be expected from single receptor cells of the various types with time. The duration of odor is indicated by the solid bar at the top of the chart, and is taken from the time of overlap of stimulation in all three types of cells. At time = 3, the 8 receptor has been narcotized and cannot respond no matter how high the concentration of olfactant. Both time and membrane concentration in arbitrary units.

TABLE 5

ODOR DIFFERENCES IN THE N-ALCOHOLS

Alcohol	δ	*Receptor stimulation sequence*
Methyl	14.5	12 - 10 - 8
Ethyl	13.5	12 - 10 - 8
Propyl	12.0	12 - 10 - 8
Butyl	11.0	12 >8 10
Amyl	10.5	10 - 12 - 8
Hexyl	10.0	8 10< 12
Heptyl	9.5	10 - 8 - 12
Octyl	9.0	10 >12 8
Decyl	8.5	8 - 10 - 12

that the first group of three alcohols all have similar stimulation patterns, while butyl alcohol, with a definite difference in odor, has the first difference in pattern. We could construct the same type of table for the n-paraffins, but since they, in contrast with the alcohols, have all approximately the same $\delta = 7$, the pattern in all cases would be 8 : 10 : 12. Our findings that the odors of the paraffins from C_2 - C_{11} are qualitatively the same is again in accord with the interpretation advanced.

The question sometimes asked is why ethyl alcohol and acetic acid, with sharp odors, produce a totally different olfactory effect when combined as ethyl acetate. Both the alcohol and the acid have values of δ near 13.5 while the ester has a value of $\delta = 9$. Physically, this decrease in cohesive energy density is a result of the great reduction in hydrogen bonding caused by eliminating the hydroxyl radicle from both the acid and the alcohol. Odor differences between the alcohol and the acid result largely from the fact that the acid has a rigid, polarizable element in its structure, the carbonyl group.

Electrophysiological studies on olfaction. As remarked previously, while direct electrophysiological evidence cannot be obtained as to the interaction of chemical substances with olfactory cells, it is relatively easy to obtain indirect evidence of the electrical changes produced in the central nervous system by olfactory stimulation. Adrian[18] has recorded the electrical activity of the olfactory bulb of the rabbit during stimulation and the records show bursts of electrical activity with each inspiration of odorous material. There is a considerable degree of specificity of the records, even though there is undoubtedly much distortion because of the relatively crude localization of the recording electrodes. From a measurement of the records obtained, it is possible to note that acetone ($\delta = 10$) produces large spikes with a total duration per inspiration of 0.1 second, and that amyl acetate ($\delta = 9$) gives large and small spikes in about equal amount and a total duration of about 0.2 second, while pentane ($\delta = 7$) gives only small spikes with a total duration of the burst of about 0.3 second. Our interpretation of these observations is that acetone enters the most rapidly into all types of receptor cells since it has a solubility parameter midway between 8 and 12. The response is largely synchronous, hence the large spikes representing summated response. Amyl acetate is a molecule closer to the paraffins than to the alcohols, and hence it can be expected to stimulate almost synchronously the 8 and 10 receptors and, asynchronously, the 12 receptors. Pentane is a substance with solubility parameter far removed from the average of all receptors, hence a lengthy and asynchronous series of impulses is to be expected. Other substances investigated lend further support to this analysis. Thus pyridine gives an electrical discharge pattern in the olfactory bulb very similar to that of acetone, and the values of δ are almost identical. Xylol and eucalyptus, with values of δ similar to amyl acetate, give similar responses, and octane, with a δ similar to that of pentane, but with a somewhat higher activity coefficient in all membranes, gives a very long discharge of small spikes. This discharge is so long as to run over into the record of the next inspiration.

We may expect, therefore, that electrophysiological investigations of the olfactory bulb, while capable of resolving the main receptor responses with respect to cohesive energy, will miss the finer discriminations that give odors their individuality because the response may be 100 to 1000 $\delta = 8$ fibers to 1 $\delta = 12$ fiber in the case of a paraffin. This difficulty is sufficiently serious to merit some special attention. If receptors of three different types are distributed randomly over the olfactory epithelium, and if there are only 10^6 receptors of each type, the probability that 3-channel recording from one receptor of each type will detect a given olfactory pattern is infinitesimal.

The nature of nervous excitation. Another difficulty in the way of understanding how olfaction takes place is our lack of information as to just how an excitable structure is altered to permit the propagation of the electrical disturbance we call the action potential. Certain features of the process are clear, while others can be inferred only from the experimental data. When an electric current of threshold strength is outwardly directed in an axon, there results under the cathode a condition of heightened excitability that, if the current flows long enough, leads to a deplorization which propagates along the axon. Presumably, the first process that occurs is the driving of certain cations, contained inside the axon, into the membrane, where they initiate structural changes (a randomization or entropy increase) which permit external Na^+ to enter. It is this local randomization of membrane structure, then, that must propagate in advance of the electrical disturbance that is observed. Since electrical stimulation is so universally used in neurophysiology, there is a tendency to disregard the fact that the stimulation of nerve can be brought about by thermal, mechanical, optical, and chemical methods. While it is entirely possible that nervous excitation can be brought about by a variety of mechanisms, it seems most probable that the feature that all of the above methods might have in common is that of randomizing the molecular structure of the membrane.

The most satisfactory model of the cell membrane that we can present at the moment is that of a surface layer of cylindrical macromolecules of diameter about 40 Å. and length 100 Å. (membrane thickness). If these molecules are arranged in regular hexagonal packing, then the interspace between any three molecules forms a "pore" of the membrane. While the "pore" is triangular in shape, a circle of diameter about 6 Å. can be inscribed in the interspace. At room temperature, the actual size of a given interspace may be expected to be subject to some dispersion, so that the membrane actually consists of a series of interspaces of varying size. In FIGURE 7 we have plotted the number of sites or interspaces versus the diameter of the site, and have shown the situation for molecules of three different sizes. The assumptions are made that a given molecule can cause narcosis if it merely occupies a sufficient number of sites, while a molecule can excite if it corresponds quite closely in size with the site it occupies. To avoid geometrical difficulties with molecules of complex shapes, the treatment outlined here is for spherical molecules only. The top curve in FIGURE 7 is for a molecule that is small as compared with the mean interspace size. The hatched area represents sites that can be occupied with a resulting narcosis, and the black bar the number of sites that, when they are occupied, can result in stimulation. The middle curve is for a molecule that corresponds in size with the mean interspace size. Only about half the sites are susceptible to narcosis, but the greatest number are potentially excitable. The bottom curve shows the situation for a large molecule: few sites for narcosis, and a small number of excitable sites.

Further analysis, using the treatment described above, shows that it will account for the shape of the curve for threshold olfaction over its entire range. A small, spherical molecule such as Xenon has no odor simply because it has a very much greater probability of finding a membrane site that is too large, rather than one of corresponding size. Very large molecules simply cannot find enough sites to occupy rapidly

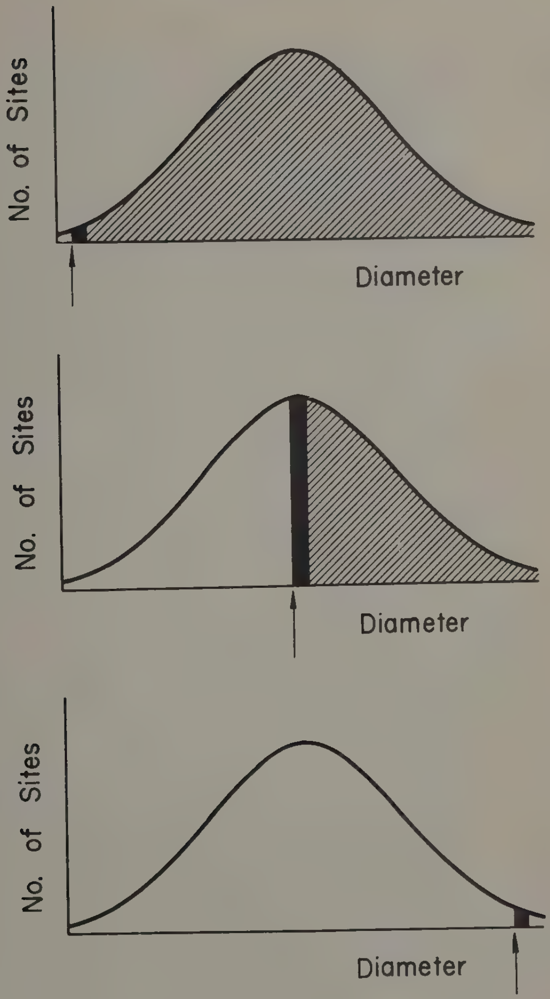


FIGURE 7. The dispersion in size of membrane sites. At top, arrow indicates the size of molecule being used for test. Black bar indicates the number of sites that are excitable by molecules of this size level, and hatching indicates the number of sites susceptible to narcosis. Middle curve, as above for molecules of size shown by arrow. Bottom curve, sites available to a very large molecule, as shown by the arrow.

enough for any olfactory activity to be initiated. This is partly because their low vapor pressure limits the number of molecules available, but mainly because of a deficiency in sites.

The fact that olfactory excitation appears so very dependent upon the shape of the molecule used for study is understandable in only a very rough way on geometrical grounds. There is no intention of minimizing the enormous complexity of the physical situation, one that can be dealt with only when our knowledge of the liquid state is further advanced. The sensitivity of a membrane to excitation should depend upon a molecule being able to create the greatest amount of membrane disorganization in the shortest possible time. The amount of disorganization produced per unit time will be related to: (1) the concentration of molecules with an optimal configuration for penetration; (2) the fraction of molecules in (1) with an orientation with respect to the cell surface such that their rate of penetration is at a maximum, (3) the structural distortion occasioned by the molecule in the membrane as a result of the altered forces between membrane molecules. In a recent study[23] it has been noted that a reasonable correlation exists between the threshold for olfaction of certain substances and the ability of these substances to accelerate the hemolysis by saponin of red blood cells. Using a Davson-Danielli model of the cell membrane, the authors suggest a "puncturing" of the membrane by foreign molecules.

According to the proposals advanced in this report, low values of A_{o1f} at a particular V_m, such as those found with some planar molecules, do not mean that a larger number of receptors are stimulated per unit of concentration. It seems more reasonable that these low thresholds result from greater simultaneity of receptor-type stimulation because of the greater ease of insertion of these molecules into the limiting receptor membranes. It is at least theoretically possible to envisage one molecule as affecting momentarily the whole membrane of one cell. There is evidence that only very small changes in membrane structure permit large changes in ion flow; 1 molecule of acetylcholine is estimated to be responsible for the flow of 30,000 sodium ions across the end plate membrane in 1 millisecond.[19] It is also generally accepted that 10^{-9} coulombs per cm^2. need to be transferred across the membrane in order that a propagated disturbance will take place. This about 10^{10} ions per cm^2. but the lower limit of size of the area that must be excited is unknown. Hensel and Zotterman[14] have shown that menthol, acting on the cold fibers of the cat's tongue, is stimulating at a concentration of $1{:}10^6$. The water solubility of menthol is 0.4 g./L. so that the activity at $1{:}10^6$ concentration is about 2.5×10^{-3}, a rather common value for olfaction. The cold fiber preparation showed further both excitation by menthol at this low concentration, and narcosis at higher concentrations. There

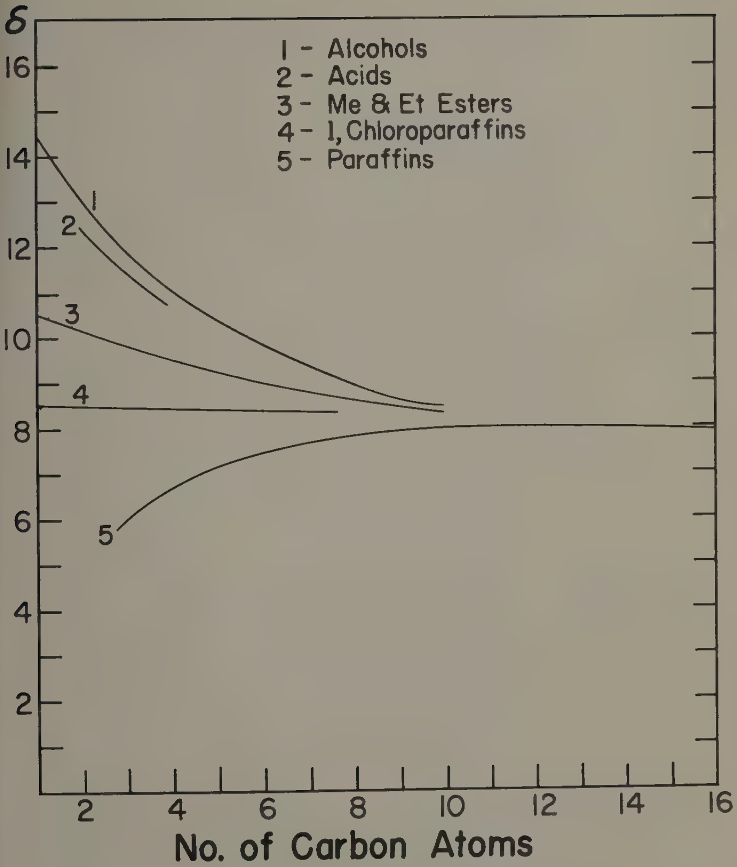


FIGURE 8. The solubility parameter of various homologous series of organic compounds is plotted against the number of carbon atoms in the molecule.

would thus appear considerable generality to the notion that many substances are capable of causing both excitation and narcosis of excitable structures, depending only on concentration.

Size and shape limits in olfaction. Certain information ought to be obtainable from an analysis of the properties of the largest molecules that can initiate olfaction. It seems clear that, in most homologous series of flexible molecules, the odor becomes more and more indefinite and difficult to describe from C_{12} on, and ceases at C_{14}. In the homologous series of cyclic ketones, however, a variety of different odors are obtained, and the series terminates with C_{16} (musk) and C_{17} (civet), both substances with quite definite and similar odors. Several factors combine to make such a difference possible. The cohesive energy density of organic compounds tends to approach a common value of about 8.5 as more and more carbon atoms are added to a molecule (see FIGURE 8), so that beyond about C_{10}, molecular size and shape are the determining factors in fixing the distribution of molecules to various receptors. Accordingly, it is to be expected that the odor of macrycyclic ring compounds is largely determined by the number of atoms making up the ring, rather than the kinds of atoms: ring ketones, imines, lactones, and carbonates with about 15 carbon atoms all have musk odors. For an aliphatic compound, the number of configurations in which the molecule can exist increases with the addition of each carbon atom, but this is not true for a ring compound that will tend to have a rather unique configuration. This rigidity of configuration will doubtless be of great assistance in making all the molecules available for penetrating into the various receptor membranes. A third factor that limits the detection of large molecules by olfaction is the ability of many substances to freeze at relatively high temperatures. It is unfortunate that physical theory is at present inadequate to account for or to predict temperatures of fusion, but since our value for A_{o1f} is defined with the liquid as the standard state, considerable deviations in activity can arise if a given substance is a solid with a high heat of fusion and a high temperature of fusion. Anthracene (C_{14}) is a rigid, cyclic molecule, but it has a rather high temperature of fusion and heat of fusion so that, at 37° C., the pure solid has an activity of only about 0.01. A very pure sample of this material that we have examined has no odor, but since threshold for this size of molecule might be expected to be $A_{o1f} = 0.1$, it may be the fact that anthracene is so far removed from the liquid state at body temperature that is responsible for its lack of odor. Musk, on the other hand, melts at about body temperature.

Summary

(1) In certain homologous series of chemically inert, flexible molecules, the values for threshold olfaction are all about the same for any given number of carbon atoms when thresholds are expressed as thermodynamic activity.

(2) A molal volume of about 40 cc./mole is a requirement for the

initiation of olfaction while 100 cc./mole appears to be optimal. Increases in threshold as one goes beyond 100 cc./mole are due to increases in activity coefficients of the molecules in the receptor membranes.

(3) Since adaptation to paraffins does not influence thresholds for alcohols and vice versa, it is concluded that there are at least two and probably more types of receptors in the olfactory epithelium. These types differ in the "cohesive energy density" or in the internal attractive forces that hold the molecules of their membranes together.

(4) The fact that all flexible molecules have roughly the same threshold for olfaction at a given number of carbon atoms is understandable if the molecules must excite simultaneously not only receptors of similar constitution, but also receptors with different attractive forces.

(5) Adaptation to odor occurs because the concentrations necessary to excite some receptor types are more than sufficient to narcotize other receptor types.

(6) Qualitative differences in odor are interpreted in the central nervous system as differences in time between the start of excitation in three receptor types. The odor of a chemical compound must be specified by the internal attractive forces, and by the size and shape of its molecules.

(7) Rigid molecules with certain shapes are very much more effective olfactory stimulants than are flexible molecules. This suggests that the excitatory action of molecules is by virtue of their ability to produce a local disorder in the oriented molecular structure of the cell membrane.

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